M. S. Mahesh · Vinod Kumar Yata *Editors*

Feed Additives and Supplements for Ruminants

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Foreword

Livestock is an integral part of the agrarian economy in most parts of the world, providing quality animal protein and thus contributing to human food and nutrition security. Owing to the greater demand for milk and meat products driven by the change in dietary preferences and increased per capita income of consumers, ruminant production has been poised to increase by >50% by the year 2050 compared to the current level. The livestock industry, therefore, aims to produce high-quality, safe animal products while duly considering factors such as consumer awareness, public health, ethical standards, and environment.

'Balanced feeding' is a key to harnessing the production potential of ruminants, and feed represents a standalone cost of about 65–70% of recurring farm expenses. While energy, protein, fat and micronutrients constitute the core components of any typical balanced diet, the feed additives and supplements occupy niche among the list of scientific interventions that has guided the enhancement of livestock production for augmenting the growing demands for animal products by the global population. However, application of some of those feed additives such as antibiotics raised grave concerns to both animal industry and public health. This has emphasised the need for developing alternate strategy to enhance the nutrient utilisation towards achieving higher productivity. Evidently, feed additives seem to be the front runner to cater to the new and emerging challenges of livestock sector. In addition to regulating rumen fermentation, feed additives help to enhance nutrient utilisation, improve metabolic functions and nutrient-use efficiency as well as the overall health status of animals. With the present emphasis on precision feeding to avoid wastage of feed, it becomes all the more relevant to provide critical nutrients and supplements as per the animal needs to have more efficient and sustainable animal production. The use of feed additives is much greater in poultry and pig production than in ruminants. However, because of its beneficial effects in improving gut health and providing proper rumen environment for augmenting better performance that translates into higher yields of saleable farm produce (milk and meat), its use in ruminants is gaining significance. In addition, usage of feed additives may become increasingly relevant in a changing climatic scenario, where livestock production is seen alongside 'efficiency' as well as 'environmental sustainability'. Besides, in practical terms, additives and supplements assist nutritionists in formulating cost-optimised diets at the farm level.

This book entitled *Feed Additives and Supplements for Ruminants* brings about in detail the applications of various additives and supplements for enhancing the productivity and profitability of ruminants. The editors have covered all the latest information on various aspects of feed additives and their relevance in present-day feeding systems. This book is very comprehensive and well-articulated. The updated information provided in this book would serve as a very useful resource material for all those interested in studying ruminant production science including researchers, nutrition advisors, veterinarians, progressive farmers, agripreneurs and feed industry personnel as well as students of animal, dairy, veterinary and agricultural sciences to enrich their knowledge in the subject and provide insights in developing economical feeding practices for farm animals.

I appreciate the editors and contributors for bringing out this valuable book pertaining to ruminant nutrition, which is very timely and need of the hour.

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Preface

'Mother cow expects from us nothing but grass and grain'

—M.K. Gandhi, Father of Nation, India

Although the above statement broadly holds good for most of the ruminants in general, the diets of modern cows with increased dairy merit demand even more than mere grass (forage/roughage) and grain (concentrates). That is how science-based feeding systems have evolved to meeting nutritional requirements in commensurate with the production performance of today's ruminant livestock.

Globally, the demand for livestock-source foods are in rise manifolds in the near future, and hence, the livestock systems—including ruminants—are transforming from traditional low-input model to advanced commercial farming. Given the genetic progress-led increased productivity of ruminants, maintaining equilibrium between the key performance indicators like production, reproduction, health and welfare has become a new challenge to ruminant producers worldwide. And, it is well-known that feeds and feeding regimen, among other factors, directly reflect the balance sheet of farms on a day-to-day basis by acting as 0.7 of standalone farm expense and thus influence sustainability on a long-run. In this context, apart from balancing the rations at farm levels for major and micronutrients, there is an increasing necessity/scope to incorporate various feed additives and supplements, which potentially help navigate with the above challenges associated with intensive farming.

The Association of American Feed Control Officials defines "additive" as 'an ingredient or combination of ingredients added to the basic feed mix or parts thereof to fulfil a specific need, and usually used in micro quantities and requires careful handling and mixing'. Unlike feed additives that are generally 'non-nutritive', the supplements are 'nutritive' in nature. Although, typically, feed additives and supplements constitute not greater than 10% of the total diet expense, their significance cannot be overlooked, owing to their myriads of health and production benefits, ultimately enabling sustainable farming. In other words, while 'supplements' may correct the underlying nutritional deficiencies of diet, 'additives' modify nutrient metabolism such that it benefits the measurable farm outcomes.

This book is a unique collection of feed additives and supplements that are systematically dealt in 23 different chapters addressing both well-known and new-generation intriguing compounds. Broadly, this book envisages production enhancement through various additives and supplements by means of rumen fermentation manipulation, immunomodulation, nutrient utilisation, cellular metabolism, etc. that ultimately dictate milk production, body weight gain, feed efficiency and reproduction. Besides, this book takes a brief discourse on improving environmental stewardship of ruminant production by minimising carbon footprint associated with greenhouse gas emissions, enhancing ruminant-derived food safety through mycotoxin binders, probiotics, exogenous enzymes, ionophores, rumen buffers, flavours and natural phytogenic (herbal) feed additives with an emphasis on plant secondary metabolites (tannins, saponins and essential oils). In addition, recent advances in nutritional supplements such as trace minerals, amino acids, B vitamins, slow-release nitrogen and functional nutrients have also been dealt in detail. Moreover, insights on feed additives for calves, transition cows as well as heat stress amelioration are covered. Furthermore, some of the lesser-known or emerging additives comprising of newer trace elements, biochar, rare-earth elements, seaweeds and silage additives constitute an additional highlight of this book. The conclusions from meta-analyses and the overview on commercial products of select additives along with cost implications of their usage further help users to get a practical outlook on the benefits of each supplement, which may also lead to new product development by the feed industry. Lastly, it may be noted that feed additives and supplements per se are not magic bullets to boost productivity; rather, when these are used judiciously in conjunction with proper ration balancing and a good feeding management, these could catalyse performance, health and profitability of ruminants.

This book holds a wide scope, and hence, it is strongly believed that it would enable worldwide readers—including researchers, animal producers, nutrition specialists, veterinarians, feed industry personnel—to appreciate the significance of proven additives and supplements in bolstering the production of global ruminant agriculture.

The authors invited to contribute the chapters are active researchers and well-qualified scientists from both academia and feed industry, who are specialists in their own specific subject domain as reflected by their quality publications in the recent past. The editors are grateful to all the authors for their quality contributions, and both the editors sincerely acknowledge the support of their respective institutions during editing of this book. Finally, we sincerely thank the publishers (Springer Nature) for their diligent efforts in bringing out the book in this shape.

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About the Book

This book is an extensive compilation of self-contained chapters on various feed additives and supplements employed in ruminant production. Broadly, this book envisages production enhancement through various additives and supplements by means of rumen fermentation manipulation, immunomodulation, nutrient utilisation, cellular metabolism, etc. that ultimately dictate performance outcomes like milk production, body weght gain, feed efficiency and reproduction. Besides, this book takes a brief discourse on improving environmental stewardship of ruminant production by minimising carbon footprint associated with greenhouse gas emissions, enhancing ruminant-derived food safety through mycotoxin binders, microbial feed additives, exogenous enzymes, ionophores, rumen buffers, flavours and natural phytogenic (herbal) feed additives with an emphasis on plant secondary metabolites (tannins, saponins and essential oils). In addition, recent advances in nutritional supplements such as trace minerals, amino acids, B vitamins, slow-release nitrogen and functional nutrients have also been dealt in detail. Moreover, insights on feed additives for calves, transition cows as well as heat stress amelioration are covered. Furthermore, some of the lesser-known or emerging additives comprising of newer trace elements, biochar, rare-earth elements, seaweeds and silage additives constitute an additional highlight of this book. The conclusions from meta-analyses and the details on commercial products and cost implications of using additives further help users to get a practical outlook on the rationale to use each additive and supplement. Overall, this book holds a wide scope and would enable worldwide readers—including researchers, nutrition specialists, veterinarians, feed industry personnel and animal producers—to appreciate the significance of proven additives and supplements in bolstering the production of global ruminant agriculture.

Features

- A comprehensive collection encompassing conventional as well as new-generation feed additives used in ruminant production
- Presents both nutritive supplements (minerals, amino acids, B vitamins, etc.) and non-nutritive additives (phytogenics, direct-fed microbials, enzymes, flavours, toxin binders, etc.)
- Holistic coverage of each additive/supplement along with their mode of action and scientifc rationale to use

- Explores specialty topics like role of feed additives in mitigating enteric methane emission, heat stress amelioration, controlling gastrointestinal parasitism and natural phytogenic additives
- Examines the role of additives and supplements in critical life stages of ruminants such as successful calf rearing and transition management
- Uses simple and lucid language, acting as a ready reference material for animal science and veterinary professionals
- Showcases practical benefits to dairy producers to enhance bottom-line profitability in ruminant farming

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About the Editors

M.S. Mahesh is an Assistant Professor (Animal Nutrition) of Livestock Farm Complex at the Faculty of Veterinary and Animal Sciences of Banaras Hindu University, Mirzapur, India. He has graduated in Veterinary Science and Animal Husbandry from Veterinary College, Bengaluru, and earned master's and Ph.D. in Animal Nutrition from ICAR-National Dairy Research Institute, Karnal, India. He was awarded with university gold medals for academic excellence during undergraduation and afterwards with Junior and Senior Research Fellowships. His areas of specialisation include use of novel feed ingredients in ruminants, feed characterisation, on-farm diet formulation and optimisation, designing species-specific micronutrient premixes, troubleshooting nutritional issues and rationalising feed additive applications for an augmented performance of dairy cattle and buffaloes. His previous association with animal feed industry helped him gain a broad-spectrum of hands-on experience on the diverse facets of practical dairy nutrition. He has published over 30 research/review papers in journals of national and international repute with a few other technical articles. He served as the former member of editorial board of *Indian Journal of Animal Nutrition* and is currently associated as a reviewer for many international journals on animal science and multidisciplinary areas. He is a life member of professional societies such as Animal Nutrition Society of India, Animal Nutrition Association, Indian Poultry Science Association and Karnataka Veterinary Council. In his present role, he teaches students and researchers on various aspects of animal nutrition.

Vinod Kumar Yata is an interdisciplinary researcher working at Center of Excellence for Aging and Brain Repair, University of South Florida (USF), Tampa, Florida, USA. Previously, he worked as a Research Associate at National Dairy Research Institute, Karnal, India. He served as an Assistant Professor at the Department of Biotechnology, Dr. B.R. Ambedkar National Institute of Technology, Jalandhar, Punjab, India. He received his Ph.D. in Biotechnology from Indian Institute of Technology, Guwahati. He specialises in interdisciplinary research which includes nanotechnology, microfluidics, animal biotechnology, cancer biology and bioinformatics. He has developed a microfluidic device for the separation of live and motile spermatozoa from cattle semen samples. His research interests have been focused on the development of nanocarriers, understanding prodrug enzyme therapy and targeted drug delivery. He elucidated the structural features and binding interactions of several biomolecules by in silico methods. He has published four books as an editor and one book as an author with Springer Nature publisher. He has published several research papers in peer-reviewed international journals and presented papers in several international conferences.

1 Dietary Applications of Exogenous Enzymes to Improve Nutrient Utilization and Performance in Ruminants

Ahmed E. Kholif and Amlan K. Patra

Abstract

Competition among animal feeds, human foods, and demands for biofuel production forces livestock producers to feed their animals on the available low-cost raw materials. Ruminant farmers provide their animals forage-based diets to reduce the cost of feeding and to keep normal rumen function. However, only forage feeding does not sometimes provide animals enough available energy and protein required for milk production and growth. The major problem with feeding low-quality forages or raw materials is their content of rigid and less-digestible cell wall components. However, ruminants have a digestive system with a complex and dynamic microbiota and microbial activities that allow them to utilize plant cell walls and degrade them to be utilized as energy sources by host. The ability of ruminants to degrade structural polysaccharides (e.g., cellulose, hemicellulose, and lignin) has a limit, and under perfect conditions, is less than 65%. Different approaches have been developed to improve nutrient digestibility and production responses in ruminants including chemical, physical, and biological strategies. Exogenous enzymes have gained an increasing interest to improve the nutritive value of ruminant feeds. The aim of administration of exogenous enzymes is to improve fiber degradation and efficiency of feed utilization

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by enhanced nutrient utilization and reduce the wastage. Exogenous enzymes are capable of breaking down specific bonds in carbohydrates, starch, protein, fats, cellulose, hemicellulose, pectin, glycoproteins, and lignin. *Lactobacillus acidophilus*, *L. plantarum*, *Streptococcus faecium*, and *Bacillus subtilis* are the main bacterial species that produce a wide array of exogenous enzymes, while *Trichoderma reesei*, *Aspergillus oryzae*, and *Saccharomyces cerevisiae* are the main fungal enzyme producers. The ability of exogenous enzymes to improve growth performance in feedlot cattle and small ruminants as well as milk production in dairy cattle has been demonstrated, but with inconsistent effects. Enzyme type and preparation, application method, level of enzyme supplementation, diets, animals and interaction between enzymes and feeds are the factors that determine the response to enzyme supplementation and are responsible for variations among experiments. This chapter reviews information about sources of enzymes, enzyme activity, modes of action, optimal inclusion, and response of ruminant to dietary applications of various enzymes.

Keywords

Animal performance · Exogenous enzymes · Feed additives · Forage fiber · Ruminants · Structural carbohydrates

1.1 Introduction

Many countries have limited resources for good-quality fodder crops. Forages are the cheapest available ruminant feeds; however, feeding animals on poor quality forages limits their performance. Poor-quality fodders do not provide enough metabolizable energy and protein intakes for optimum milk production and daily gain in ruminants due to their high contents of structural fibrous carbohydrate components, e.g., cellulose, hemicellulose, and lignin (Taghizadeh and Nobari 2012). Furthermore, slow and/or incomplete digestion of fiber in diets limits their total tract digestion, especially when forages or fibrous feeds are the major component in the diets.

Animal nutritionists have explored new techniques to increase the digestibility of low-quality fibrous feeds (Elghandour et al. 2014; Azzaz et al. 2020; Simon et al. 2024). Different approaches including physical, chemical, and biological treatments of forages were employed to achieve these goals. The supplementation with exogenous enzymes showed mixed results with milk and feedlot performance; however, improved feed utilization and performance were observed in most studies. The reasons for the administration of exogenous enzymes in ruminant diets include: (1) to increase the availability of nutrients enclosed within fiber-rich cell walls; (2) improving the capability of ruminal endogenous enzymes to breakdown specific chemical bonds in raw materials; and (3) to break down antinutritional factors in feeds. The improvements in performance and nutrient utilization with feeding exogenous enzymes observed in many studies with cattle (Tewoldebrhan et al. 2017), goats (Azzaz et al. 2020), sheep (Salem et al. 2015a), and buffaloes (Morsy et al. 2016) encouraged their commercial applications. Exogenous feed enzymes increase the digestibility of nutrients, feed efficiency, minimize the environmental impact in animal industry by allowing better use of natural resources, and reducing wastage (Kholif et al. 2017b).

The first investigations on enzyme administration in animal feeds were performed in early 1920s; however, the first commercial production of enzymes using submerged fermentation systems in large fermenters was started in the 1950s. At that period, responses were variable and modes of action were not clear. The enormous development in fermentation technology allowed for the economic production of large quantities of enzymes with high biological activities to be used as livestock feed additives to manipulate the digestive processes in ruminants. Most enzyme applications in ruminants have focused on the use of polysaccharide-digesting enzymes to improve the ruminal degradation of the relatively slowly digested fiber. Cellulases and xylanases are the two major types of enzymes commonly considered to improve fiber digestion. Cellulases are complex mixtures of proteins that act to hydrolyze β-(1-4)-glucosidic linkages, which connect the individual glucose molecules in the cellulose found in forages. Xylanases degrade xylans in the hemicellulose fraction in forages. The main methods of application of exogenous enzymes in animal feeding are the pretreatment of feeds before feeding or the direct feeding enzyme preparations to improve the degradation activities in the gastrointestinal tract of animals. Thus, microbiologists and nutritionists recommend the addition of active enzyme preparations to ruminant diets prior to feeding. This chapter provides some recent information demonstrating the important effects of specific enzyme preparations on microbial activities in the rumen and discusses their impact on the performance and nutrient utilization in ruminants.

1.2 Cell Wall Components

The maturity of the forages or the amount of lignification can influence the composition and structure of plant polysaccharides and cell wall components. Each plant cell contains a tough and rigid layer consisting mainly of cellulose, hemicellulose, lignin, and pectin, which provides the cell with physical and structural support and protection. The cell wall primarily contains three major polymers including: cellulose at 35–50%, xylan (hemicellulose) at 20–35%, and lignin at 10–15% (Mendoza et al. 2014). The cellulose microfibrils are linked via hemicellulosic tethers to form the cellulose-hemicellulose network, which is embedded in the pectin matrix. Xyloglucan is the most common hemicellulose in the primary cell wall (Mendoza et al. 2014). Cellulose is a linear molecule of insoluble β-glucans, comprising of D-anhydro-glucopyranose residues linked by β(1-4) bonds (Paloheimo et al. 2010). Cellulose is present in the cell walls of plants, trees, tunicates, algae, and several species of bacteria as exopolysaccharide membranes (Lynd et al. 2002; Khandelwal and Windle 2013). The cellulose composition differs between microorganisms and plants (Lin et al. 2013). With hemicellulose and lignin, cellulose forms a cellulosic biomass complex.

Hemicellulose has a substantial role in maintaining cell wall structure in plants. It accounts for about a quarter of total plant biomass (Scheller and Ulvskov 2010). Hemicellulose is a heterogeneous group of polysaccharides characterized by β-(1-4) linkages, which includes xyloglucans, glucuronoxylans, glucuronoarabinoxylans, glucomannans, and galactoglucomannan (Scheller and Ulvskov 2010). The main component in hemicellulose is xylan.

Lignin is a macromolecule composed of highly cross-linked phenolic molecules (Cosgrove 2001). There are different forms of lignin including guaiacyl, 5-hydroxygualacyl, p-hydroxyphenyl, and syringyl lignin (Moore and Jung 2001). The concentration of lignin varies depending on the nature of the plant species, maturity, soil and environmental conditions, organs, and cell wall layers (Mendoza et al. 2014). Lignin has a high molecular weight that limits the availability of the structural carbohydrates to rumen microorganisms (Van Soest 2019), which, in turn, limits the digestibility and overall forage nutrient availability to ruminants.

Additionally, cell wall contains structural proteins at about 1–5%, which are classified as hydroxyproline-rich glycoproteins and arabinogalactan proteins, glycine-rich proteins, and proline-rich proteins (Moire et al. 1999). The concentrations of all components in the cell wall differ between plants, cell type, and cell age (Taghizadeh and Nobari 2012).

1.2.1 Ruminal Degradation of Cell Wall Components

Ruminants have their digestive system for efficient utilization of plant cell walls. Under optimal conditions, cell wall digestibility in the total gastrointestinal tract is less than 65% (Van Soest 2019). Ruminants have a compartment known as 'rumen', which is a very efficient fermentation system that houses a vast array of different microbes. The rumen microbial ecosystem shows high resilience and inertia properties due to core microbiota composition and also has great plasticity owing to other parts of the microbiota (Patra 2020). The consortial activities of the ruminal microbiota render ruminants to utilize complex forages including browse forages/foliages, grasses, and other plant materials due to the ability to degrade and ferment carbohydrates in plant cell walls and provide volatile fatty acids and protein to the host animals. Taghizadeh and Nobari (2012) determined four factors controlling fiber degradation in the rumen: (1) the structure and composition of ingested plants; (2) densities of the predominant fiber digesters in the rumen; (3) microbial factors regulating microbial attachment to feed particles and hydrolysis; and (4) animal factors that increase the availability of nutrients through mastication, salivation and digesta kinetics.

Ruminants digest cellulosic feeds mainly in the rumen by the microbiota. The environment in the rumen is anaerobic and contains dense populations of several species of bacteria (10–50 × 10^9/mL), protozoa (10^5–10^6/mL), archaea (10^7–10^8/mL), bacteriophages, fungi (10^4–10^5/mL), and sometimes yeasts (Russell and

Rychlik 2001; Patra 2020). *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* are the main bacterial species with a high fibrolytic activity (Koike and Kobayashi 2009). *Butyrivibrio fibrisolvens* produces cellulases; however, it has a more predominant function in the hydrolysis of hemicellulose, and therefore, has a central role in fiber digestion (Devillard et al. 2003). *F. succinogenes* works synergistically with noncellulolytic bacteria to digest parenchyma bundle sheaths, epidermal cell walls, and leaf sclerenchyma (Akin 1989). *Prevotella ruminicola* is not a highly cellulolytic bacterium, but produces a range of xylanases. Rumen also contains a number of less well-characterized cellulolytic bacteria such as *Eubacterium cellulosolvens* (Krause et al. 2003).

Ruminal protozoa contribute about 19–28% of total ruminal cellulolytic activity (Gijzen et al. 1988), and therefore, defaunation may reduce fiber digestion (Bonhomme 1990; Ushida and Kojima 1991). On the other hand, defaunation may increase the numbers of ruminal bacteria and fungi including fiber-degrading enzyme activities. Also, defaunation increases the requirement for non-protein nitrogen, which partially explains the reduction in fiber digestion (Ushida and Kojima 1991). The concentration and composition of the protozoal fauna depend upon many factors including composition of diet, ruminal pH, turnover rate, frequency and level of feeding. Diets with 40 and 60% concentrate support the maximal protozoal numbers (Dehority and Orpin 1997). *Isotricha*, *Dasytricha*, *Metadinium*, *Diplodinium*, *Eudiplodinium*, *Ophryoscolex*, *Entodinium*, and *Epidinium* are the major genera of ciliate protozoa (Dehority and Orpin 1997). Ruminal protozoa are extremely sensitive to thermal shock, pH, and exposure to oxygen (Dehority and Orpin 1997).

Fungi contributes approximately 8% of the microbial biomass in the rumen (Orpin and Joblin 1997). The anaerobic fungi in the rumen may have an important role in fiber digestion due to their ability to penetrate both cuticle and cell wall of lignified tissues and to degrade recalcitrant cell wall materials, including the sclerenchyma and vascular tissue (Orpin and Joblin 1997). Ruminal fungi solubilize plant fibers more efficiently than cellulolytic bacteria (Joblin et al. 1989). The high cellulase and hemicellulase activities in fungi can be improved by hydrogen utilizing methanogens (Joblin et al. 1989), which decrease the repressive effect of hydrogen (Orpin and Joblin 1997). The main reason for the low number of ruminal fungi is the slow generation time compared to ruminal bacteria (6–9 h *vs.* 0.5–3.5 h, respectively) (Taghizadeh and Nobari 2012). *Neocallimastix* spp. are the well-studied ruminal fungi with a high activity against crystalline cellulose and can degrade more than 34% of the plant fibers (McSweeney et al. 1994).

The rumen contains a complex community of fibrolytic microorganisms that catalyze the degradation of fiber in the rumen. Fibrolytic enzymes produced in the rumen are mainly divided into cellulolytic, hemicellulolytic, and pectinolytic (Wang and McAllister 2002). Xylanases are the main enzymes degrading xylan core polymers into soluble sugars. Endoglucanases hydrolyze cellulose chains and produce cellulose oligomers including cellobiose, which is hydrolyzed by β-glucosidases to glucose (Beauchemin et al. 2003). Xylanases and β-1,4 xylosidase are the main enzymes involved in degrading the xylan core and convert it into soluble sugars

(Bhat and Hazlewood 2009). The xylanases mainly include endoxylanases, in addition to other hemicellulases including β-mannosidase, α-L-arabinofuranosidase, α-D-glucuronidase, α-D-galactosidase, and acetyl xylan esterases (Bhat and Hazlewood 2009).

1.3 Exogenous Enzymes in Ruminant Nutrition

Exogenous enzymes in ruminant feeding can be divided mainly into three categories including fibrolytic, amylolytic, and proteolytic enzymes. Based on the site of action, endo- and/or exo-acting enzymes are available (Zhang and Lynd 2004). Fibrolytic enzymes hydrolyze cellulose, hemicellulose, and pectin. Endocellulase, exocellulase, and β-glucosidase are the main cellulose hydrolyzers, while endo-β-1,4-xylanases and β-1,4-xylosidases are the main hemicellulose hydrolyzers. Mendoza et al. (2014) defined a set of enzymes that hydrolyze cellulose into simple units: endoglucanases, exoglucanases, and β-glucosidases. Endoglucanases (EC.3.2.1.4) hydrolyze cellulose chains randomly to produce oligomers of cellulose, while exoglucanases (EC.3.2.1.91/176) hydrolyze cellulose to cellobiose. β-glucosidases (EC3.2.1.21) release glucose from cellobiose. Xylanase hydrolyzes the 1,4-β-D-xylosidic linkages in xylans, which are constituents of hemicellulose (Karamian and Ranjbar 2011). Other enzymes such as acetyl xylan esterase, ferulic acid esterase, α-D-glucuronidase, α-L-arabinofuranosidase also play important roles in the fibrolytic activity.

Another method of enzyme classification is based upon the types and activity of enzymes produced, the strain selected for production, the substrate used for production, and the conditions of production (e.g., culture) (Gashe 1992; Lee et al. 1998).

1.3.1 Common Feed Enzymes for Ruminants

Ruminants typically consume large amounts of forages rich in cell wall fractions (40–70% of total forage dry matter) with complex networks, which limit their optimal degradation and energy availability (Elghandour et al. 2016; Kholif et al. 2017b). However, exogenous enzymes offer a potential to digest such feeds. Although thousands of different enzymes have been discovered, only a few of them have been used as feed enzymes (Fig. 1.1). Broadly, feed enzymes can be divided into three main categories: amylolytic enzymes, proteolytic enzymes, fibrolytic i.e., β-glucanase, xylanase, and β-mannanase (McCleary 2009).

Carbohydrases are a group of enzymes that hydrolyze carbohydrates such as starch and fiber components. Smaller carbohydrates are broken down into simple monosaccharides by the intestinal glucoamylase, sucrase, isomaltase, and lactase, but complex fiber carbohydrates such as cellulose and hemicellulose are not hydrolyzed by pancreatic or intestinal enzymes but require some enzymes produced by microorganisms within the gastrointestinal tract. Such enzymes have the ability to effectively breakdown the β-glycosidic bonds in the fiber, making them easier to

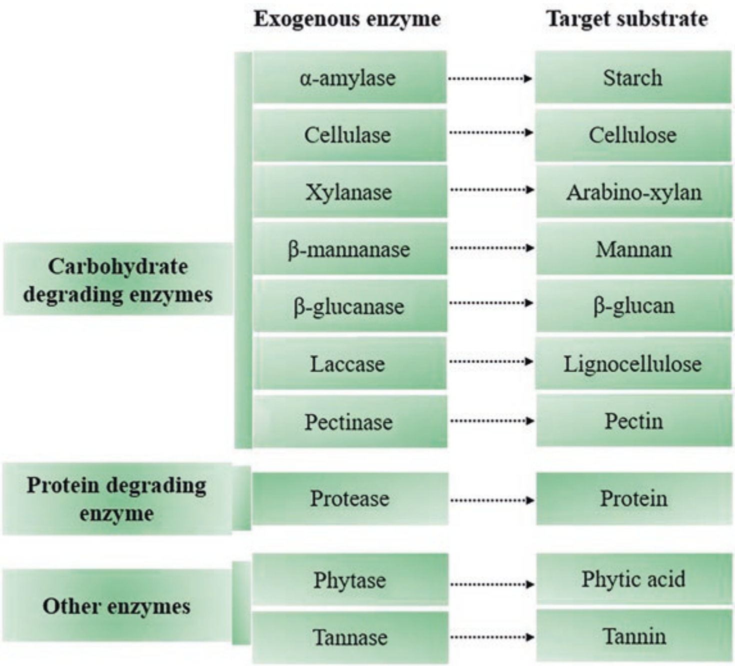

Fig. 1.1 Exogenous enzymes explored in ruminant nutrition

digest in the rumen and also have beneficial impacts in the small intestine (Fontes et al. 1995). Xylanases and β-glucanases are the main fiber-degrading enzymes used in animal feeds. Xylanases breakdown arabinoxylans present in grains and their by-products. While β-glucanases attack β-glucans in feeds and plant by-products, amylases act on starch granules in grains and their by-products. Xylanases and β-glucanases are commonly fed alone (Togtokhbayar et al. 2015; Vallejo et al. 2016), while amylases are rarely fed alone but usually as a part of an enzyme mixture containing xylanases, glucanases, proteases, and phytases.

Lignolytic enzymes are a set of exogenous enzymes produced by white-rot basidiomycetes, which produce relevant and unique oxidative lignolytic extracellular enzymes, making them the strongest decomposers of lignocellulosic biomass (Eichlerová et al. 2006; Kholif et al. 2014). The white-rot basidiomycetes produce peroxidase or phenoloxidase that help in the mineralization of lignin and produce highly reactive radicals oxidizing both the phenolic and non-phenolic lignin components (Sridhar 2017). Laccases are the primary set of lignolytic enzymes belonged to the oxidase family. Laccases are copper-containing enzymes that catalyze the oxidation of various substrates with the simultaneous reduction of molecular oxygen to water (Sridhar 2017). The fungal laccases are mostly inducible and extracellular glycoproteins with carbohydrate contents of 10–20%, which may contribute to the high stability of laccases. These enzymes have been used in many experiments to delignify a wide range of crop residues like wheat, paddy, barley, mustard straws, as well as alfalfa-grass hays (Sridhar et al. 2015; Thammiaha et al. 2016).

Amylolytic enzymes have a great role in diets rich in cereal grains (e.g., corn and barley). Pérez et al. (2009) stated that amylolytic enzymes can produce as much as 600 metabolizable glucose units by targeting α-glycosidic bonds and increasing starch digestion (Rojo et al. 2007). Tricarico et al. (2008) hypothesized that amylolytic enzymes work by cross-feeding mechanisms of ruminal bacteria through oligosaccharides produced by the enzymes, creating modified products of ruminal fermentation. Further, supplementation of amylolytic enzymes to ruminants showed mixed results. Hristov et al. (1998) observed no effect on ruminal or total-tract digestibility in lactating dairy cattle fed high-grain diets supplemented with polysaccharide-degrading enzymes. Conversely, DeFrain et al. (2005) in steers and Tricarico et al. (2005) in lactating dairy cattle observed increased proportions of acetate and propionate with exogenous α-amylase supplementation.

Proteases are a set of enzymes that catalyze the hydrolysis of proteins into peptides and amino acids throughout the rumen and small intestine. The major role of proteases is to hydrolyze trypsin inhibitors and lectins (these factors can inhibit digestion) and improve protein digestibility. Their use is not common in ruminant nutrition (Devant et al. 2020) because abundances of endogenous proteolytic enzymes are produced by the ruminal microorganisms. However, proteolytic enzymes can target nitrogen cross-linkages in the cell wall of forages resulting in increasing the digestibility of fibers and can also provide additional N to digestible N pool and increase the total amount of fermentable organic matter in the rumen (Colombatto and Beauchemin 2009). Feeding lactating Holstein cows on high- or low-forage diets supplemented with exogenous proteolytic enzymes increased digestibility of neutral detergent fiber, acid detergent fiber and hemicellulose in enzyme-supplemented low forage diets (Eun and Beauchemin 2005). It has been noted that the main problem with feeding exogenous proteases is increasing the concentration of ruminal ammonia nitrogen (Eun and Beauchemin 2005).

Phytase plays a critical role in the digestion and availability of phosphorus in feeds (El-Hack et al. 2018). Plants contain sufficient amounts of phosphorus to meet animal requirements of phosphorus; however, animals do not utilize them optimally because about 60–80% of total phosphorus is phytate-bound (NRC 2001), making it indigestible, thus reducing its absorption along with other nutrients and trace minerals (Ghosh et al. 2016; Long et al. 2017). Ruminal microbiota produce phytase to make available phosphorus for absorption, but grain processing, passage rate, and dietary calcium concentrations could reduce the ability of endogenous phytase to release phosphorus from phytate (Long et al. 2017). Therefore, phytase can help in the availability of P in feeds (Knowlton et al. 2007), although its supplementation has been studied in limited extent. Supplementation of exogenous phytase to high sorghum grain diets reduced fecal phosphorus excretion and tended to increase neutral detergent fiber digestibility in Rambouillet rams (Vallejo et al. 2018), but no effects on feed intake, dry matter digestibility, and phosphorus balance were noted in feedlot cattle (Long et al. 2017).

1.3.2 Production/Sources of Feed Enzymes

There are many preparations of enzyme products available in markets for livestock. Generally, commercial enzymes available for livestock feed industry are produced from microbial batch fermentation process, starting with a seed culture and growth media (Taghizadeh and Nobari 2012). Four bacterial species (*Bacillus subtilis*, *Lactobacillus acidophilus*, *L. plantarum*, and *Streptococcus faecium*) and three fungal (*Aspergillus oryzae*, *Trichoderma reesei*, and *Saccharomyces cerevisiae*) species are the main producers of enzymes for feed industry (Taghizadeh and Nobari 2012; Singh et al. 2018). Living organisms do not produce only single enzyme (e.g., cellulases or hemicellulases), but produce combinations of several enzymes such as amylases, proteases, or pectinases. Enzyme characteristics, activities, and composition differ depending on the microorganisms, strain selected, growth substrate, and culture conditions even within a single microbial species (Khattab et al. 2019). As a number of enzymes with relative proportions and activities are required to degrade cellulose and hemicellulose, combining a diversity of enzyme activities in the same preparation is advantageous. It ensures that a wide variety of substrates can be targeted by a single product. For the commercially available enzyme products, crude enzyme extracts at specified levels of one or two defined enzyme activities, such as xylanase and/or cellulase, are blended. Successful enzyme products should: (1) have high activities; (2) have high level of resistance to inactivation by heat treatment, low pH and proteolysis; (3) be produced at cheaper cost; and (4) have a long shelf-life under ambient storage conditions.

The enzyme production occurs using fermentation processes that can be classified into: solid-state fermentation and submerged fermentation or liquid fermentation. The difference is mainly based on the type of substrate used during fermentation. In the solid-state fermentation, the fermentation takes place in a slow and steady manner with a very long period of fermentation and leads to controlled release of nutrients. While the solid-state fermentation uses solid substrates such as bran and bagasse, the submerged fermentation uses liquid substrates like broth and molasses with a rapid production of enzymes (Satapathy et al. 2018). The latter is recommended for the production of exogenous enzymes from bacteria due to the higher requirement of water, whereas the solid-state fermentation is recommended for the production from fungi.

More than 75% of the industrial enzymes are produced using submerged fermentation utilizing genetically modified organisms. Cellulases and xylanases are produced using both solid-state fermentation and submerged fermentation. Production of cellulases and xylanases from bacteria has some advantages such as the high growth rate of bacteria that can sustain in highly variable environment and provide better activities (Sujani and Seresinhe 2015). On the other hand, cellulases and xylanases produced from fungi have some advantages such as the capability to produce ample amounts of enzymes, easy extraction, and purification (Satapathy et al. 2018).

1.3.3 Modes of Action of Exogenous Enzyme

The physiological responses to exogenous enzyme supplementation are multifactorial, making it a very complicated process. The overall effects of enzyme supplementation may be a result of their ability to expose slowly degraded substrates to microbial attack. It is likely that exogenous enzymes act in the rumen shortly after feeding and during a period just before bacterial colonization and prior to the time when naturally occurring microbial enzymes are produced and initiate fiber digestion. The exogenous enzymes complement the endogenous enzyme systems associated with the fibrolytic activities of the microorganisms in the rumen and allow for greater digestion of fibrous substrates during the initial critical stages of preliminary digestion.

The mode of exogenous enzymes includes a number of effects on the gastrointestinal microbiome and the ruminant animals itself. Generally, exogenous enzymes affect feed utilization via the direct hydrolysis of the feeds, enhancing the attachment of ruminal microbes to feed particles, changing gut viscosity, and synergism with ruminal enzymes. McAllister et al. (2009) suggested three possible mechanisms for the effects of exogenous enzymes on feed digestion and animal performance: pre-consumption effect, ruminal effect, and post-ruminal effect.

1.3.3.1 Pre-consumption Effect

The treatment of feeds with exogenous fibrolytic enzymes before feeding (e.g., silages and other conserved forages) releases reducing sugars partially from the solubilization of fiber fractions prior to consumption and increases the available carbohydrates in the rumen (Lynch et al. 2014; Togtokhbayar et al. 2015). This effect shortens the lag time needed for microbial colonization and accelerates microbial attachment and growth (Forsberg et al. 2009). The degree of sugar releasing depends upon both the type of feeds and enzyme used (Singh et al. 2018). Moreover, the pretreatment with fibrolytic enzymes removes structural barriers that limit ruminal microbial digestion of the feeds.

1.3.3.2 Ruminal Effects (Synergistic Action)

Synergism between the endogenous and exogenous enzymes for feed digestion occurs in the rumen (Singh et al. 2018).The effect of ruminally unprotected exogenous enzymes will depend upon the stabilization since they are usually rapidly degraded in the rumen similar to dietary proteins (Morgavi et al. 2001). The stabilization of exogenous enzymes in rumen depends on the origin and type of enzyme activity (Morgavi et al. 2001). Wallace et al. (2001) observed increased xylanase activity by 5% and cellulase activity by 15% when these exogenous fibrolytic enzymes were administered directly in feeds at 1.5 mg/g diet, suggesting the stability of exogenous enzymes in the rumen.

The stability of exogenous enzymes and the synergy with the endogenous enzymes in the rumen have been reported. For example, the net combined hydrolytic activity of the exogenous and endogenous enzymes in the rumen is greater than that of a single source (Newbold 2007). Feeding exogenous enzymes increased the number of total and cellulolytic microbes in the rumen (Newbold et al. 1992). Giraldo et al. (2008) noted increased number of ruminal microbes and fibrolytic activity (mainly xylanases activity) when a high forage diet was treated with exogenous enzymes.

Ruminal pH is an important factor that affect the activity of exogenous enzymes in the rumen. Under low rumen pH (<5.9), effectiveness of exogenous enzymes is lower than at higher rumen pH conditions (Beauchemin et al. 2004). At low ruminal pH, the adhesion of microbial cells to their feed particles decreases (Spanghero et al. 2003), which is the first step in the efficient digestion of feed substrates in the rumen. Colombatto et al. (2007) also observed greater degradation of fiber components at higher ruminal pH. Usually, ruminal pH close to neutrality is recommended for the maximum hydrolytic activity of exogenous enzymes.

1.3.3.3 Post-Ruminal Effects

Exogenous enzymes may change the site of nutrient digestion and increase fibrolytic activity in the small intestine (Hristov et al. 2000) or reduce viscosity of intestinal digesta (McAllister et al. 2009). Reducing digesta viscosity could improve the absorption of nutrients in the small intestine, especially with feeding of grain-rich diets (Singh et al. 2018). In the small intestine, exogenous enzymes appear to act for a sufficient period of time with sufficient effects on substrate particles when applied to wet feeds and concentrate premix (Morgavi et al. 2001; Beauchemin et al. 2004). Additionally, Beauchemin et al. (2004) stated that the remaining exogenous enzymes may work synergistically with microbes in the large intestine.

1.3.4 Inclusion Levels of Exogenous Enzymes

One of the primary reasons that affect the response of exogenous enzyme supplementation is the appropriate dose of specific enzyme preparations for specific substrates (Colombatto et al. 2003; Togtokhbayar et al. 2015). This explains the importance of enzyme-feed specificity and presents a major dilemma for formulating new ruminant feed enzyme products. The application of exogenous enzymes at a low dose may not exploit its optimum hydrolytic potential, while their overdose of exogenous enzymes may lead to molecular crowding, leading to decreased feed intake and digestibility (Bommarius et al. 2008) and can depress microbial activities. Although the reason is not clear; however, it may reflect the abilities of certain enzymes, at high levels, to depress the normal enzyme systems produced by the ruminal microbial population. Both in vitro and in vivo studies suggest that the responses to different levels of enzyme supplementation are not linear and that optimal activities of specific enzyme preparations occur over narrow ranges of inclusion rates.

Beauchemin et al. (2003) noted that high doses of enzyme administration can be less effective than low doses. Other researchers (Nsereko et al. 2002) observed a quadratic response in total bacterial numbers in ruminal fluid with increasing levels of an enzyme product from *T. longibrachiatum* added to a dairy cow diet. Low and moderate doses of enzyme cause disruption of the surface structure of the feeds; however, increasing the enzyme levels diminishes the disruption of the feed surface structures (Nsereko et al. 2002). Excess exogenous enzyme attachment to feed particles may restrict microbial colonization and limit digestion of feeds (Morgavi et al. 2000). Morgavi et al. (2000) observed a stimulated adhesion of *Fibrobacter succinogenes* to corn silage and alfalfa hay with low level of fibrolytic enzymes from *T. longibrachiatum*, while increasing enzyme levels competed with the rumen bacterium for available binding sites on cellulose.

Three explanations can interpret the negative effects associated with increasing the level of enzyme supplementation: (1) increasing enzyme levels may reduce the amount of neutral detergent fiber available for fermentation, causing reduced microbial protein and volatile fatty acid production; (2) the presence of bound enzymes may restrict the access of microorganisms to the feeds; and (3) high levels of enzymes may release anti-nutritional by-products such as phenolic compounds, which inhibit microbial growth.

In ruminants, the diets typically contain several types of forages and concentrate mixes, and therefore, it would be advantageous to include a number of different enzyme sources. Due to the difficulty to achieve this goal, it is recommended to use an enzyme product that may not be the best on all forages, but is relatively suitable for most feeds (Beauchemin et al. 2003). This is also the main reason to use multi-enzyme products, also known as enzyme cocktail, in ruminant diets (Azzaz et al. 2020).

1.3.5 Application Methods of Exogenous Feed Enzymes

Methods of enzyme delivery is an important factor that should be considered to maximize the benefits of using fibrolytic enzymes in dairy diets. There are several methods to administer exogenous enzymes in ruminants; however, the most effective method is yet to be recognized. The most widely used application methods include the pretreatment of the feeds before feeding (e.g., during silage making and forage harvesting), the application at the time of feeding (in hay, in total mixed rations, or in concentrate), or the direct infusion to the rumen (Beauchemin et al. 1995; Feng et al. 1996; Rode et al. 1999).

Yang et al. (2000) noted that the addition of fibrolytic enzyme to concentrates for 1 month before feeding increased diet digestion and milk production in dairy cows. Rode et al. (1999) observed better responses to liquid enzyme application to the concentrate or hay than when applied to a total mixed ration before feeding. However, Sutton et al. (2003) stated that the application of exogneous enzymes directly to the total mixed ration is recommended for better responses compared to the application into the concentrate or directly infusing to the rumen. Indeed, applying exogenous enzymes to a large portion of the diets increases the chances of enzymes to endure in the rumen (Beauchemin et al. 1999a).

The administration of liquid enzymes to dry feeds increases their adsorption to feed particles and increases the resistance of exogenous enzymes to ruminal proteolysis and prolong their viability in the rumen (Beauchemin et al. 1999b; Taghizadeh and Nobari 2012). Also, mixing exogenous enzymes with their substrates would increase their chances of bypassing the rumen and tends to increase their enzymatic activities (Fontes et al. 1995). Moreover, applying enzymes to feeds prior to feeding alters the structures of the chemical compounds in feeds, thereby making it more susceptible to degradation, which ultimately result in enhanced ruminal fiber digestion (Elghandour et al. 2016). In addition, the favorable responses were observed when enzymes were applied to high-moisture feeds such as silages compared to dry feeds because the higher moisture content helps in the hydrolysis of soluble sugars from complex polymers (Beauchemin et al. 2003). Exogenous fibrolytic enzyme administration in silage provides more fermentable sugars for silage fermentation and improves silage dry matter digestibility (Kung et al. 1991; Ridla and Uchida 1999). Hvelplund et al. (2009) observed that the application of exogenous enzymes during ensiling increased neutral detergent fiber content and decreased the in vitro neutral detergent fiber digestibility compared to the silage without enzyme application. However, Lynch et al. (2014) observed unchanged nutritive value of alfalfa with exogenous fibrolytic enzyme during ensiling. This is more suitable method for exogenous enzyme supplementation because farmers store and process their forage and grains prior to feeding.

The exogenous enzymes administered directly before feeding may be released into the ruminal fluid and may pass quickly before they can be effective in the rumen (Khattab et al. 2011). Exogenous enzymes increase microbial attachment of ruminal microbes to feeds and increase activity of enzymes associated with feed particles (Wang et al. 2001).

In other experiments, researchers observed that infusing exogenous enzymes directly into the rumen is less effective than direct application to feeds (Lewis et al. 1996; McAllister et al. 1999; Wang et al. 2001). Hong et al. (2003) compared enzyme supplementation methods (applied to rumen or enzyme-treated diet) with another treatment without enzymes and observed that enzyme application method did not affect ruminal fermentation, enzyme activity, and total-tract apparent digestibility. However, they reported increased nutrient degradation rate and effective degradability of dry matter and fiber components with increasing pretreatment times. It was assumed that the unchanged enzyme activity in the rumen indicates that exogenous enzyme was not destroyed by ruminal proteases or that the endogenous enzyme activities in the rumen was high enough, and therefore, exogenous enzyme did not add any extra benefit (Hong et al. 2003). These authors further stated that the effects of enzyme supplementation were mostly intestinal and a significant proportion of exogenous enzymes escaped ruminal digestion and remained active in the small intestine. Elghandour et al. (2016) compared the addition of xylanase directly before fermentation or with a 72-h pre-incubation before fermentation of maize stover, oat straw, and sugarcane bagasse. They observed that the preincubation of substrates with enzymes was more effective in improving the fermentation compared to the direct addition of enzyme just prior to fermentation. Therefore, the method of enzyme application becomes an important factor determining the positive responses of enzyme supplementation to the diets of ruminants.

1.3.6 Exogenous Enzymes and Performance Attributes of Ruminants

Many factors determine the response of ruminants to enzyme supplementation and thus performance outcome. Physiological status of animals, nutrient requirements, method of exogenous enzyme application, diet, nature, and activity of enzymes, as well as levels and mode of enzyme supplementation are the main factors. Additionally, the energy status of animal is another prime factor (Yang et al. 2000). These factors partially explain the inconsistencies and variations of results reported in the literature.

1.3.6.1 Effects on Ruminal Microbial Activities and Fiber Disappearance

The ability of exogenous enzymes to affect the solubilization of some types of dietary fiber and to induce some changes in ruminal microbial activities are the most important response to exogenous enzyme administration (Wang and McAllister 2002; Sujani and Seresinhe 2015; Singh et al. 2018; Tang et al. 2023). In many experiments, the changes in ruminal microbial activities have been demonstrated in batch cultures of ruminal bacteria by measuring the individual volatile fatty acid production rates and by stoichiometrically estimating the amount of carbohydrates that are degraded to produce these volatile fatty acids (Wang and McAllister 2002).

Studies with xylanase-rich enzyme preparations enhanced the production of total volatile fatty acids and overall carbohydrate utilization by ruminal microbial population, enhanced dry matter, and fiber digestion (Vallejo et al. 2016). Exogenous enzyme preparations rich in cellulases cause dramatic changes in the physiological activities of the ruminal bacteria resulting in a more efficient fermentation (Wang and McAllister 2002). Certain types of exogenous enzyme preparations cause dramatic effects on the overall ruminal fermentation activity (Kholif et al. 2018).

One of the main reasons for enzyme administration in ruminant is to breakdown the fibrous matrix in plant structural carbohydrate complexes and to expose many of the bound nutrients to digestive activities in the rumen. In a meta-analysis, Tirado-González et al. (2018) summarized the results of 586 articles published during 2000–2012 and reported improvements in the in vivo dry matter and neutral detergent fiber digestibility with the administration of exogenous enzymes. Exogenous enzymes render the fiber soluble and increase their availability for ruminal microbial attack, especially during the first 6–12 h of the digestive process. Moreover, supplementation of exogenous enzymes increases the initial rate of dry matter disappearance and/or the rate of neutral detergent fiber disappearance. Also, the initial rate of fiber digestion is an important factor affecting feed intake, especially for forage-based diets (Van Soest 2019). The enzyme activity can be influenced by the nature of fiber, age and maturity of the plant materials, nutritional status of the animals, and the types of enzyme systems provided in the supplement, making it difficult to predict the responses to many types of enzyme preparations.

1.3.6.2 Effect on Feedlot Cattle

Performance of feedlot cattle depends upon feed availability in different geographical regions. For example, these cattle are mainly fed on grass in countries with excess or low value lands, while finish cattle are fed with a grain ration in countries with excess feed grain resources. In many areas and due to economic issues, producers also feed their animals on by-products rich in pectin or starch compounds. Therefore, exogenous enzymes supplementation can play important roles in feedlot operation. Limited information is available on the efficiency of exogenous enzyme supplementation at different nutritional stages (i.e., starter, grower, and finisher). Some research evaluated the ability of exogenous enzymes to enhance the utilization of high-forage diets (Krueger et al. 2008; Álvarez et al. 2009).

Starch represents the major component in diets of finishing cattle; therefore, any improvement on starch digestion in the rumen may improve productivity. However, fibrolytic enzymes have been given focused attention in most of the research in ruminant nutrition compared to those involved in the process of starch digestion. Thus, exogenous amylases have received little attention because ruminants extensively digest starch, which does not limit production. Moreover, accelerating starch digestion in the rumen may cause ruminal acidosis (Abdela 2016). However, the supplementation with dietary α-amylase to finishing diets may result in increased feed intake and daily gain (Tricarico et al. 2007).

Responses to exogenous enzyme supplementation varied with positive, negative, or no effects. In a meta-analysis, Tirado-González et al. (2018) observed that exogenous enzyme supplementation to diets containing <50% grasses increased daily body weight gain. Moreover, they observed positive effects on feed conversion efficiency in animals fed low-forage diets and in diets containing ≥50% grasses, but no positive effects on daily gain and feed conversion in the high-forage diets. Balci et al. (2007) reported that supplementing diets with exogenous enzymes at 60 g/day improved daily weight gain, total weight gains, and feed conversion rates in Holstein steers. Krueger et al. (2008) observed increased dry matter intake and neutral detergent fiber digestibility without affecting final body weight, average daily gain, and body condition score in steers fed Bermudagrass supplemented with fibrolytic enzymes. Gómez-Vázquez et al. (2011) concluded that increasing the supplementation rate of exogenous enzymes (0, 15, or 30 g fibrolytic enzyme/kg concentrate) linearly increased daily gain, intake, digestion, and feed conversion. Carcass characteristics with exogenous enzymes (fibrolytic or amylolytic) supplementation to Angus yearling bulls were evaluated by Neumann et al. (2018). It was found that the fibrolytic enzymes increased average daily gain and gain: feed ratio, while exogenous amylolytic enzymes increased total carcass weight, rib-eye area, and marbling (Neumann et al. 2018). Recently, Lourenco et al. (2020) observed that the inclusion of endo-1,4-β-xylanase and endo-1,3(4)-β-glucanase into creep feeds increased average daily gain and gain:feed ratio in calves. Vargas et al. (2013) observed minimal effects on body weight gain, feed conversion efficiency with improved carcass characteristics, and tenderness of steers fed a diet containing 88% concentrate and 12% forage and additionally supplemented with fibrolytic enzymes at 0, 2, 4, and 6 mg/kg for 75 day. Conversely, Russell et al. (2016) reported decreased neutral